

PROJECT REVIEW

“Improved detection of Oral-Turinabol structure identification and elimination of metabolites and generation of reference material”

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As per list of the World Anti-Doping Agency (WADA) 2015 Oral-Turinabol (4-chloro-17 β -hydroxy-17 α -methylandrosta-1,4-dien-3-one), which was extensively misused by GDR athletes is prohibited in sports. In recent years it has been rediscovered by producers of "dietary supplements" and gained new importance in doping control (continuously increasing numbers of adverse analytical findings since 2003). Following its administration long-term metabolites with 4-chloro-17 β -hydroxymethyl-17 α -methyl-18-norandrost-13-ene structure have been identified. However these metabolites are not available as reference substance up to now. Even though the use of post-administration urines instead of purified reference material has been accepted in confirmatory analyses, the athletes in question may challenge the results of the anti-doping laboratories resulting in prolonged trials. The goal of the present project is to produce Oral-Turinabol long-term metabolites with 4-chloro-17 β -hydroxymethyl-17 α -methyl-18-norandrost-13-ene structure that cannot be synthesised via classical chemical synthesis. Therefore a joint chemical synthesis and biotechnological approach will be applied. The substrate (17,17-dimethyl-18-nor-13-ene intermediate) will be converted by single-step hydroxylation to the desired product in a whole-cell biotransformation assay using recombinant strains of fission yeasts that express the human cytochrome P450 enzymes CYP 3A4 or CYP 21 as already successfully performed for the analogue metandienone long-term metabolite by our group, that is now available in worldwide anti-doping laboratories. As already published by our group both enzymes catalyze the respective reaction in Oral-Turinabol as well. Prior to the biotechnological hydroxylation, the substrate will be chemically derived from Oral-Turinabol by Wagner-Weerwein rearrangement. Purification and subsequent NMR structure confirmation will be performed and the product distributed to the anti-doping community.